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Calculation of relative free energy via indirect pathways

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A general method is presented to reduce the simulation time required to compute the relative free energy between two states X and Y of a molecular system by computer simulation. Although the free energy difference $\Delta A_{x\to y}$ is, in principle, independent of the pathway chosen to change X into Y, in practice its choice strongly affects the accuracy of the obtained ΔA value. The optimum path is the one for which the relaxation time of the system τ_{system} attains a minimum, allowing the system to remain as close as possible near equilibrium during a simulation. Downscaling the relevant parts of the potential energy function before the change from X to Y is made, and upscaling afterwards is a rather general way to shorten τ_{system} and thus save computing time. For a model system of butane like molecules the proposed procedure is more than 1 order of magnitude more efficient than the conventional technique of direct interconversion from state X to state Y.

I. INTRODUCTION

There are many examples currently in the literature of attempts being made to calculate the relative free energy between two molecular systems by way of molecular dynamics (MD) or Monte Carlo (MC) computer simulation.[1-6] Potentially, this is one of the most useful applications of these techniques. The rational design of new drugs and the prediction of the relative stability of molecular structures depend on knowledge of the free energy of the system. While many thermodynamic properties can be calculated as statistical equilibrium averages from a MD trajectory or MC ensemble, the entropy of the system and the associated free energy are global properties and as such dependent on the extent of available phase or configurational space. As in a normal case, it is not practicable to search all configuration space; direct determination of the total free energy of the system is not generally possible. The free energy change associated with a small change or perturbation to such a system can, however, be calculated during the course of a simulation. This fact forms the basis of the so-called coupling parameter approach for determining the free energy difference between two molecular systems with different interaction potentials. The change in free energy between two systems X and Y can be determined from a series of MD or MC simulations at intermediate values of the coupling parameter λ, yielding derivatives of the free energy with respect to λ.

Alternatively, a single MD simulation can be carried out in which the potential energy function V is slowly changed such that the system changes from X to Y over a reversible path. The free energy is determined as the work necessary for such a change. The method strongly depends, however, on the system always remaining close to equilibrium and a representative region of configurational space being sampled at each point on the path between X and Y. Failure to meet these conditions is often manifested as a disparity between the free energy change in the forward and reverse process. Statistical effects resulting from poor averaging can, of course, be reduced by repeating, or increasing the length of, the simulation. In contrast, the effect of varying the potential energy function V faster than the relaxation rate of the system such that the system no longer remains close to equilibrium will result in a systematic under- or overestimation of the free energy associated with the change. This is the origin of the hysteresis between the results for the forward and reverse reactions commonly observed in dynamical simulations. Not only does this situation lead to considerable uncertainty in the final result but it can also be easily misinterpreted. This point has recently been discussed in some detail[7] including the extreme case where the system is mutated so rapidly that it effectively remains frozen in the starting configuration during the entire forward and reverse simulation process leading to apparently accurate but, in fact, entirely spurious results.

Being a state function, the free energy difference between two systems is independent of the path chosen to travel between them. Nevertheless, it is commonly assumed that the most direct path, one in which the free energy changes linearly in going from the initial to the final state as a function of a coupling parameter as illustrated by pathway I in Fig. 1, or the path along which the smallest incremental changes in free energy are obtained, is also the most efficient. A pathway like path II in Fig. 1 is thought to be less efficient since the incremental free energy change is larger than along pathway I. This ignores the fact that the allowed rate of change of the potential energy function V is governed by the condition of equilibrium, and so by the relaxation rate of the system in response to the change in V being made. The total time required to traverse the indirect path II from X to Y reversibly may indeed be less than that required using the direct path I if the relaxation time of the system τ_{system} via pathway II is significantly shorter than τ_{system} for pathway I.

In this paper we demonstrate that it is in fact possible to significantly reduce both the total simulation time required

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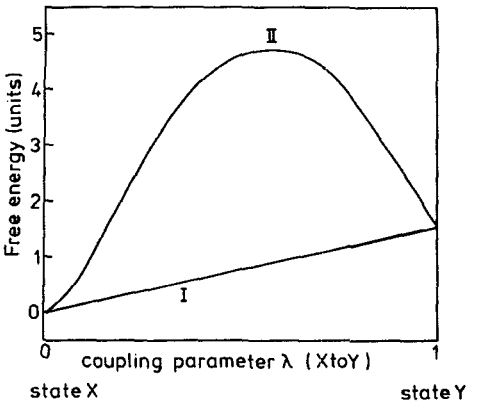


FIG. 1. Schematic drawing of a change in free energy by changing a molecular system from state X to state Y along two different pathways I and II using the coupling parameter approach.

and the observed hysteresis by the use of alternative pathways which while being indirect, permit a more rapid relaxation of the system. Further insight is also presented into the nature of the hysteresis and implications of a particular choice of the starting configuration.

II. METHODOLOGY

The simplest way to increase the rate at which configurational space is sampled, and to increase the overall relaxation rate is to raise the simulation temperature. A path can be envisaged in which the temperature T_1, of the system in state $X(T_1)$ is first increased to give state $X(T_2)$ with T_2 which is then mutated to state $Y(T_2)$ at the same temperature, T_2. The temperature of state $Y(T_2)$ is then reduced to the original temperature, T_1, to give state $Y(T_1)$:

$$\begin{array}{lccc} & T_2 & \text{—} & \text{—} \\ \text{temperature} & & \uparrow & \downarrow \\ & T_1 & \text{—} & \text{—}. \\ & & X & Y \\ & & \text{state} & \end{array} \tag{1}$$

The mutation from X to Y in such a scheme is carried out while the system is in a high state of mobility at temperature T_2, which facilitates the sampling of configurational states allowing the process to be conducted more rapidly than at the original temperature T_1. The total free energy change from state X to Y, however, now becomes a sum of the free energy changes in each of the three stages.

It is possible to treat a temperature perturbation in an analogous fashion to interaction perturbations[8] and to derive an expression for the change in free energy going from T_1 to T_2 from the fundamental expression for the Helmholtz free energy A

$$A = -k_B T \ln Z, \tag{2}$$

where k_B is Boltzmann's constant and T is the absolute temperature. Z is the canonical partition function expressed in terms of the Hamiltonian $H(p,q)$ which gives the total energy of the system in terms of (generalized) coordinates q and momenta p of the atoms of the system. For a system of N atoms we can write

$$Z = [h^{3N}N!]^{-1} \int\int \exp[-H(p,q)/k_B T]\, dp\, dq, \tag{3}$$

where h is Planck's constant and the factor $N!$ represents a shorthand notation for the number of permutations of indistinguishable particles, i.e., if there are m types of indistinguishable particles, the factor $N!$ has to be replaced by $[N_1!N_2!\cdots N_m!]$, where N_i denotes the number of particles of type m. From Eqs. (1) and (2) it can be readily shown[3] that

$$\frac{\partial(A/T)}{\partial(1/T)} = Z^{-1}[h^{3N}N!]^{-1} \int\int H(p,q) \times \exp[-H(p,q)/k_B T]\, dp\, dq \tag{4}$$

so that

$$\frac{\partial(A/T)}{\partial(1/T)} = \langle H(p,q)\rangle_T. \tag{5}$$

Integrating Eq. (4) we find

$$\frac{A_2}{T_2} - \frac{A_1}{T_1} = \int_{T_1}^{T_2} \langle H(p,q)\rangle_T d\left(\frac{1}{T}\right). \tag{6}$$

This integral can readily be evaluated during the course of a molecular dynamics simulation where the reference temperature of the heat bath is slowly altered.[9] The coupling parameter approach yields for a change from system $X(\lambda = 0)$ into system $Y(\lambda = 1)$ at constant temperature[1,2]

$$A(Y) - A(X) = \int_{X(\lambda=0)}^{Y(\lambda=1)} \left\langle \frac{\partial H(p,q,\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda. \tag{7}$$

Using Eq. (6) in the first and last stage of scheme (1) and using Eq. (7) in the middle stage the desired free energy difference becomes

$$\begin{aligned} &A(\text{state Y}, T_1) - A(\text{state X}, T_1) \\ &= T_1 \left\{ \int_{T_1}^{T_2} \langle H\rangle_{T,\lambda=0} d\left(\frac{1}{T}\right) \right. \\ &\quad + T_2^{-1} \int_0^1 \left\langle \frac{\partial H}{\partial \lambda} \right\rangle_{T_2,\lambda} d\lambda \\ &\quad \left. + \int_{T_2}^{T_1} \langle H\rangle_{T,\lambda=1} d\left(\frac{1}{T}\right) \right\}. \end{aligned} \tag{8}$$

The main disadvantage of this approach is that in raising the temperature the entire system is affected. It is not possible to selectively heat only the region of the system that is to be mutated or the degrees of freedom that are involved in the mutation. Thus, in large systems a significant degree of error could be introduced evaluating the first and last integral in Eq. (8) which would contain large and canceling terms involving changes irrelevant to the desired mutation. A second less important one is that the maximum integration step size that can be used in a simulation is smaller the higher the temperature of the system. Therefore, we propose a different approach.

The population of states of a system in equilibrium is governed by the Boltzmann factor

$$e^{-H(p,q)/k_B T} = e^{-K(p)/k_B T} e^{-V(p,q)/k_B T}, \tag{9}$$

where $V(p,q)$ represents the potential energy, and $K(p)$ the kinetic energy. This means that scaling the temperature up-

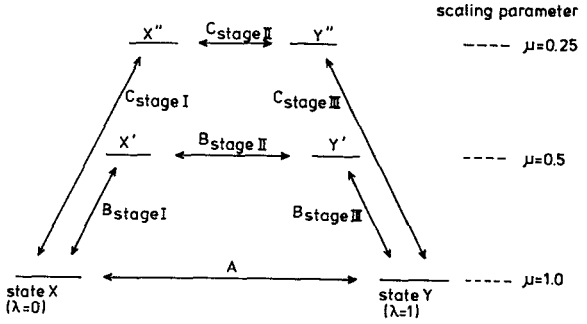


FIG. 2. Different pathway to change a molecular system from state X to state Y using the coupling parameters μ and λ according to Eq. (17).

ward by a factor μ is equivalent to scaling the potential energy V down by a factor μ. For the Hamiltonian

$$H(p,q,\mu) = K(p) + \mu V(p,q), \tag{10}$$

if we only consider changes in which the total number of atoms remains unchanged, we find like in Eq. (7)

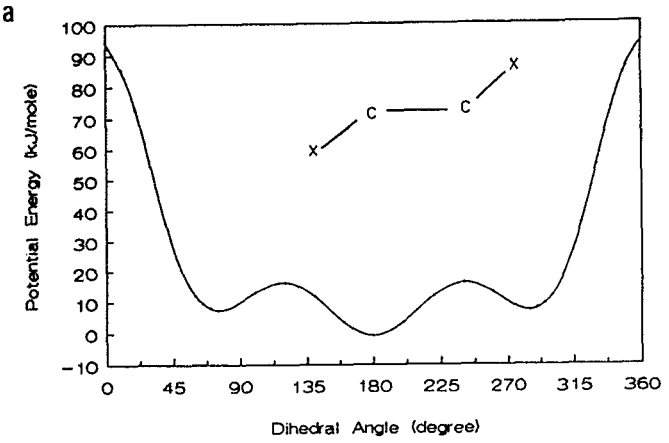
$$A(\mu_2) - A(\mu_1) = \int_{\mu_1}^{\mu_2} \langle V(p,q) \rangle_\mu \, d\mu, \tag{11}$$

which may be straightforwardly evaluated from a molecular dynamics simulation. The overall scheme thus becomes as sketched in Fig. 2. Stage I: scale the potential of the system V by a factor μ. As with increasing the temperature, lowering V lowers both the difference in potential energy between states and the potential energy barriers, resulting in more efficient sampling of conformational space. Stage II: while V is low mutate the system with respect to a coupling parameter λ to give the desired final state but with low overall potential energy. Stage III: rescale V to give the final state at normal potential energy. The sum of the free energy contributions for each of the three stages then gives the total free energy change from the initial to the final state. The advantage of this approach is that only the components of V that are directly related to the perturbation from X to Y need to be scaled.

The method proposed here is reminiscent of umbrella sampling methods,[10] in which an additional umbrella potential is added to facilitate equilibration between different regions of configurational space. Umbrella sampling techniques are normally used to derive potentials of mean force with respect to a predefined system coordinate, but they could be applied as well to the present case of modification of the interaction potential. A rapidly equilibrating pathway between X and Y could be constructed by adding artificial potentials at intermediate points. The method proposed here is a convenient special case of this general idea with the advantage that no information is needed on the potential of intervening states. A technical difference is that only a simple scaling of the potential is involved in our method.

III. MODEL SYSTEM

The sketched approach has been tested by determining the free energy change associated with the mutation of molecule X(X–C–C–X), shown in Fig. 3(a) to molecule Y(Y–C–C–Y) shown in Fig. 3(b). The potential field for each molecule was chosen such that molecule X preferentially adopts a *trans* conformation whereas molecule Y is predominantly *gauche*. The potential energy functions V for both molecules as a function of the dihedral angle (ϕ), describing rotation around the central bond are also given in Figs. 3(a) and 3(b) for molecules X and Y, respectively. The force field was constructed using an intermolecular Lennard-Jones term, an intramolecular 1-4 Lennard-Jones term and terms for the bond angles θ and dihedral angle φ and has the form[11]



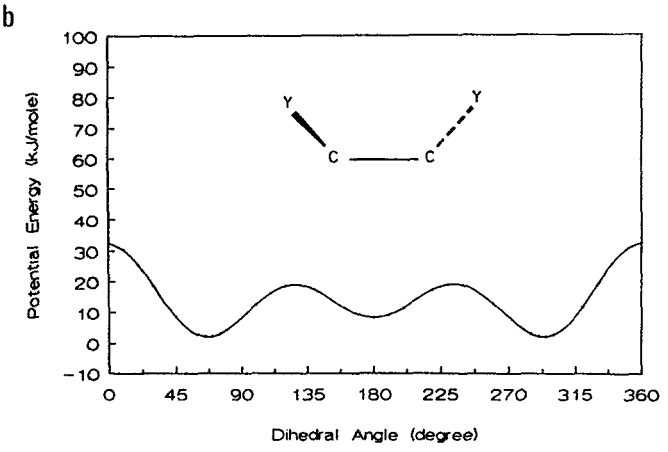


FIG. 3. Intramolecular part of the potential energy function for a four atom molecule as a function of the dihedral angle: (a) in state X (predominantly *trans*) (b) in state Y (predominantly *gauche*).

$$V(\mathbf{r}_1,\mathbf{r}_2,\ldots,\mathbf{r}_N) = \sum_{\text{angles}} 1/2K_\theta(\theta - \theta_0)^2 + \sum_{\text{dihedrals}} K_\varphi[1 + \cos(n\phi - \delta)] + \sum_{\text{pairs}(i,j)} (C_{12}/r_{ij}^{12} - C_6/r_{ij}^6). \tag{12}$$

Bond lengths were constrained at all times to 0.153 nm using the SHAKE algorithm[12] and a geometric tolerance of 10^{-4}.

TABLE I. Interaction parameters.

(A) molecule X (X–C–C–X)

Intermolecular Lennard-Jones			
	C_6 (kJ mol^{-1} nm^6)		C_{12} (kJ mol^{-1} nm^{12})
C C	$0.909\,75\times10^{-2}$		0.3533×10^{-4}
C X	$0.898\,63\times10^{-2}$		0.3040×10^{-4}
X X	$0.887\,65\times10^{-2}$		0.2615×10^{-4}
Intramolecular 1-4 Lennard-Jones			
	C_6(kJ mol^{-1} nm^6)		C_{12}(kJ mol^{-1} nm^{12})
X X	$0.685\,26\times10^{-2}$		0.1206×10^{-4}
Bond angles			
	K_θ (kJ mol^{-1} rad^{-2})		θ_0 (degrees)
X–C–C	460.24		111.0
Dihedral angle			
	K_φ (kJ mol^{-1})	n	δ (degrees)
X–C–C–X	8.368	3	0.0
X–C–C–X	0.0	2	0.0
Mass			
X : 15.035 amu			
C : 14.027 amu			

(B) Molecule Y (Y–C–C–Y)

Intermolecular Lennard-Jones			
	C_6(kJ mol^{-1} nm^6)		C_{12}(kJ mol^{-1} nm^{12})
C C	$0.909\,75\times10^{-2}$		0.3533×10^{-4}
C Y	$0.898\,63\times10^{-2}$		0.3040×10^{-4}
Y Y	$0.887\,65\times10^{-2}$		0.2615×10^{-4}
Intramolecular 1-4 Lennard-Jones			
	C_6(kJ mol^{-1} nm^6)		C_{12}(kJ mol^{-1} nm^{12})
Y Y	0.1674×10^{-2}		0.1506×10^{-5}
Bond angles			
	K_θ (kJ mol^{-1} rad^{-2})		θ_0 (degrees)
Y–C–C	460.24		111.0
Dihedral angles			
	K_φ (kJ mol^{-1})	n	δ (degrees)
Y–C–C–Y	8.368	3	0.0
Y–C–C–Y	4.184	2	0.0
Mass			
Y : 15.035 amu			
C : 14.027 amu			

The parameters used during the simulations are given in Table I. A cutoff radius of 0.8 nm was applied to the intermolecular interactions. For the mutation from molecule X into Y only the 1-4 Lennard-Jones and the dihedral angle terms were altered. The system parameters were chosen such that molecule X is comparable to *n*-butane. The *trans* conformation has, however, been stabilized relative to the *gauche* conformation and the barrier to rotation going from *trans* to *gauche* has been increased from 14 to 18 kJ mol^{-1}. This barrier is equivalent to approximately 7 times kT at 300 K.

For any system, the free energy difference, ΔA between two states X and Y is given by

$$\Delta A = A_X - A_Y = -k_B T \ln \frac{Z_X}{Z_Y}, \tag{13}$$

where again Z denotes the partition function. If bond lengths and bond angles are held constant, the partition function for both molecules can be expressed in terms of the single main degree of freedom, i.e., rotation around the central bond. If metric tensor effects[13] are neglected for sake of simplicity, the partition function may be approximated by

$$Z_i = C_i \int_0^{2\pi} d\phi \exp[-V_i(\phi)/k_B T], \tag{14}$$

where ϕ is the dihedral angle. The term C_i represents other components of the partition function such as packing and solvent effects. As in this example bond lengths, intermolecular interactions, and bond-angle forces were not changed changing V from X to Y, it may be assumed to a first approximation that $C_X \simeq C_Y$. Thus the free energy difference between molecules X and Y can be approximated by

$$\Delta A_{X-Y} \approx -k_B T \ln \frac{\int_0^{2\pi} d\phi\, e^{-V_X/k_B T}}{\int_0^{2\pi} d\phi\, e^{-V_Y/k_B T}}. \tag{15}$$

Substituting Eq. (12) for V and using the parameters in Table I we obtain a value of $\Delta A_{X-Y} = 1.4$ kJ mol^{-1}, which may be used for comparison with the results from the simulations.

In assessing the method we propose to compute free energy differences, we have compared the relative efficiency for the mutation of molecule X to Y in terms of total simulation time vs the observed hysteresis in the free energy, for three possible pathways (A, B, and C) shown diagrammatically in Fig. 2. Pathway A represents the direct mutation of X to Y by linearly scaling the total potential (1-4 interaction and torsional angle interaction).[2] The effective potential V_{eff} experienced by the molecule can be written as

$$V_{\text{eff}} = V_X(1-\lambda) + V_Y\lambda, \tag{16}$$

where the coupling parameter λ is changed linearly from 0 to 1 during the course of the simulation. B and C represent alternative pathways. These may be expressed in terms of a second coupling parameter μ which acts by scaling the effective potential such that

$$V_{\text{eff}} = \mu[V_X(1-\lambda) + V_Y\lambda]. \tag{17}$$

Pathways B and C can be considered to have three distinct stages. In the first stage all interactions in the system are scaled by μ with λ held constant at zero. Second, λ is changed linearly from 0 to 1 with μ held constant. Finally, all interactions in the system are rescaled to normal with λ held constant at 1. In pathway B μ ranged from 1.0 to 0.5 whereas in pathway C μ ranged from 1.0 to 0.25.

All simulations were performed using a cubic box (length of edges equal to 2.00 nm) containing 64 identical molecules at constant volume with periodic boundary conditions. The temperature of the system was held constant by weak coupling[14] to an external bath of 300 K using a temperature relaxation time $\tau = 0.1$ ps. The MD time step was 2 fs. The free energy in all cases was traced using the thermodynamic integration method as implemented in the GROMOS87 molecular simulation package.[11]

IV. RESULTS

A. Pathway A

Table II shows the effect of increasing the simulation time on the calculated free energy. The error associated with the average is given as half the hysteresis between the forward and reverse processes. Figure 4 shows the trace of ΔA as a function the coupling parameter λ for two simulation

TABLE II. Free energy associated with the mutation of molecule X to Y (forward) and molecule Y to X (reverse) via path A in Fig. 2 carried out at various rates.

Time (ps)	Forward (kJ mol^{-1})	Reverse (kJ mol^{-1})	Average[c] (kJ mol^{-1})
10	6.84[a], 6.39[b]	−3.58	1.52 ± 5.1
20	5.34[a], 6.30[b]	−2.85	1.48 ± 4.3
50	5.00[a], 4.67[b]	−1.52	1.66 ± 3.2
100	3.08[a], 4.06[b]	−0.83	1.37 ± 2.2
200	3.17[a], 3.09[b]	0.17	1.65 ± 1.5
500	2.93	0.51	1.72 ± 1.2

[a] Starting configuration was *trans* 58, *gauche* 6 molecules.
[b] Starting configuration was *trans* 60, *gauche* 4 molecules.
[c] The error is half the hysteresis between the forward and reverse process.

lengths, i.e., 100 and 500 ps. The upper two lines represent the change from molecule X to Y with the lower two broken lines representing the reverse process from Y into X. The central solid line has been calculated using Eq. (15) expressed in terms of the coupling parameter λ. For convenience the curves are presented arbitrarily referenced to zero when $V_{\text{eff}} = V_X$. The time over which the coupling parameter λ was changed from 0 to 1 is indicated adjacent to each curve. As expected increasing the total simulation time leads to a gradual convergence between the forward and reverse processes towards the theoretical (gas phase approximation) curve. Nevertheless, even when the mutation is made over a total period of 500 ps, a simulation time, which is much longer than normally available when dealing with macromolecules, a substantial hysteresis is still observed. This is due to the slow relaxation of the system. The curves shown in Fig. 4 represent an average over 64 molecules. The fluctuations in free energy due to contributions of individual molecules are thus greatly reduced. Despite this we still do not observe a completely smooth transition curve from X to Y. The statistical scatter which results from poor sampling, is particularly significant when considering macromolecular systems where normally only a single molecule is treated.

In order to rationalize why 500 ps is insufficient to obtain a reliable estimate of the overall free energy change which is only of the order of 1.4 kJ mol^{-1} and where the height of the intervening barrier is less than 3.0 kJ mol^{-1}, as determined from Eq. (15), it is necessary to consider in more detail the interconversion process between X and Y.

From the Boltzmann factor (9) we can estimate that the equilibrium ratio of *trans:gauche* rotamers for molecule X is approximately 60:4 and for molecule Y it is approximately 3:61. Obviously, as the molecules are mutated one into the other the populations of the *trans* and *gauche* forms must redistribute. Thus, the rate at which the system will respond

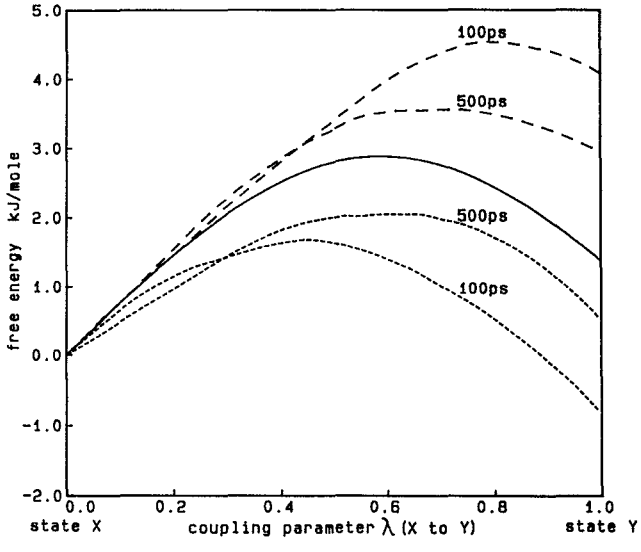


FIG. 4. Plot of the relative free energy as a function of the coupling parameter of λ for the direct mutation of molecule X to molecule Y (– – –) and molecules Y to X (---). The total simulation time is indicated next to each curve. The solid line represents an analytical approximation calculated from the (gas phase) partition function using Eq. (15). For convenience all curves have been referenced to $\Delta A = 0$ when $V_{\text{eff}} = V_x(\lambda = 0)$.

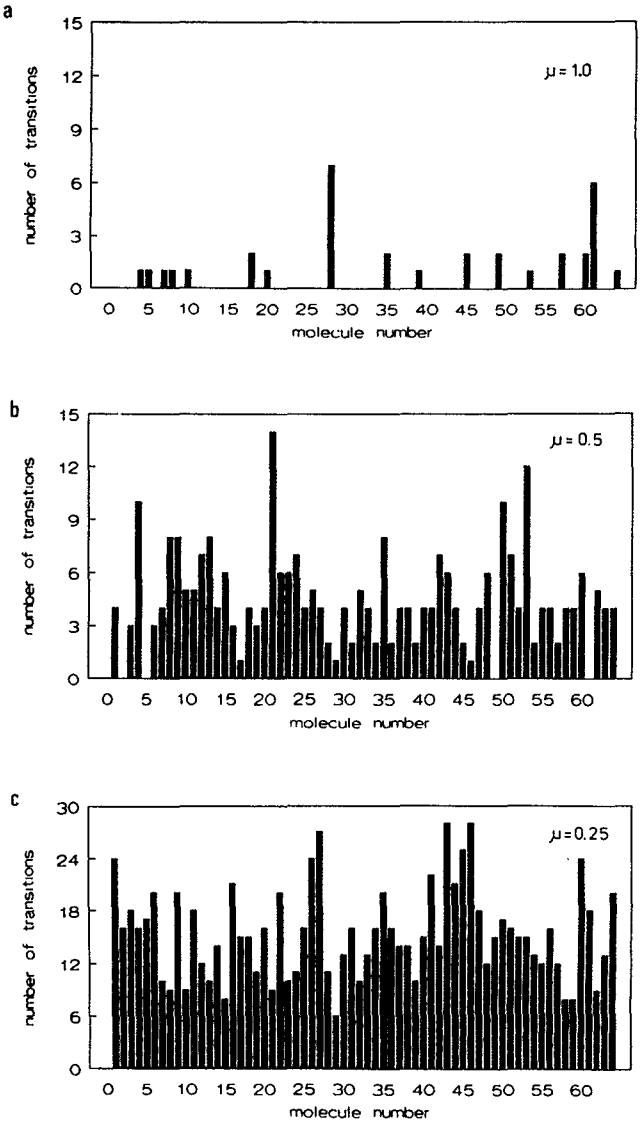


FIG. 5. The number of dihedral angle transitions as a function of molecule number over 20 ps for an equilibrated liquid containing 64 molecules of type X using $\lambda = 0$ and different values of the coupling parameter μ in Eq. (17): (a) $\mu = 1.0$, (b) $\mu = 0.5$, (c) $\mu = 0.25$.

to the change in potential will be primarily determined by the frequency of dihedral angle transitions. Figure 5(a) shows the number of dihedral transitions over 20 ps as a function of molecular number for molecule X. On average each molecule underwent one transition every 40 ps during the simulation. It should be noted, however, that the transitions were not evenly distributed; 38% of all the observed transitions occurred in just two molecules. The system, therefore, will respond very slowly to the change in effective potential necessitating long simulation times to ensure a reversible path. For example, in the extreme case shown in Table II where λ was changed from 0 to 10 ps it is obvious that the system will have remained close to the initial configuration and a meaningless value for the free energy was obtained.

B. Pathways B and C

We now consider the alternative pathways labeled B and C in Fig. 2. Both pathways consist of three stages. The initial and final stages involve the scaling of the potential. In the middle stage the actual mutation between molecule X and Y occurs. Considering this middle stage first we can readily see from Fig. 6, which shows the free energy for the forward and reverse reactions as a function of λ for path B ($\mu = 0.5$) and path C ($\mu = 0.25$), that scaling down the potential energy dramatically decreases the time required for the reversible interconversion of X and Y.

When $\mu = 0.5$ it is possible to reversibly convert X to Y in 100 ps with a negligible hysteresis for the forward and reverse processes. When $\mu = 0.25$ the same process requires just 50 ps. The effect of μ may be rationalized in terms of an increase in the overall mobility of the system. Figures 5(b) and 5(c) show the number of dihedral transitions over a 20 ps period again as a function of molecular number for molecule X but with $\mu = 0.5$ and $\mu = 0.25$, respectively. The effect of progressively scaling down all interactions has been to increase the frequency of transitions from 1 every 40 ps, to 1 every 4.5 ps when $\mu = 0.5$, to 1 every 1.3 ps when $\mu = 0.25$. There is also progressively a more even spread of the transitions over the molecules compared with the case of $\mu = 1.0$ shown in Fig. 5(a). Yet it should be noted that even when $\mu = 0.5$, 3 of the 64 molecules only show a single transition and 4 show no transitions at all over a 20 ps period.

As a final point in regard to Fig. 6 it should be noted that although all contributions to the potential energy were scaled equally it should not be expected nor is it observed that the free energy scales linearly with μ. The free energy associated with stage II of path B or stage II of path C cannot be directly related to the free energy difference as λ is changed 0 to 1 via path A. Only the sum of the three stages can be compared to the results from path A.

C. Scaling the potential

The pathway still critically depends on being able to scale the potential reversibly. The free energy associated with scaling the potential for molecule X from $\mu = 1$ to 0.5 can be seen from Table III to be in the range of 7.3 kJ mol^{-1}. This is several times the total free energy change via pathway A. Nevertheless, by comparing the values for the forward and reverse processes over 10, 20, and 50 ps shown in Table III, it can be seen that the process is in fact highly reversible, the hysteresis over 50 ps being 0.16 kJ mol^{-1}. A similar conclusion can be drawn for the process of scaling the potential for molecule Y where the observed hysteresis is in fact smaller. The reason why it is possible to reversibly scale the potential so rapidly, even though it is associated with a large free energy change, can be understood be examining the equilibrium configuration of the system when $\mu = 1.0$ and that when $\mu = 0.5$. As stated previously we can estimate from the Boltzmann factor (9) that the ratio of *trans* to *gauche* rotamers for molecules for type X is approximately 60:4 when $\mu = 1.0$. When μ is changed to 0.5 the ratio *trans*:*gauche* is expected to exhibit a minor change to approximately 46:18. Second, as shown in Fig. 5 the probability that transitions will occur increases as μ is lowered. For molecule

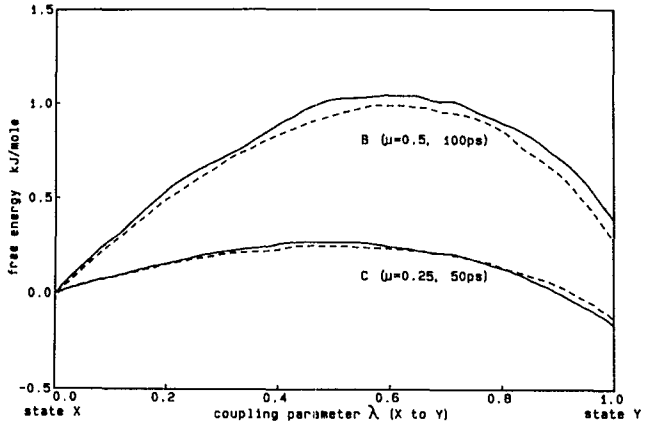


FIG. 6. Relative free energy as a function of the coupling parameter λ corresponding to stage II in pathways B and C as shown in Fig. 2. The solid lines indicate the mutation from X to Y and the broken line the reverse mutation from Y to X. The curves labeled B correspond to path B where the simulations took 100 ps with $\mu = 0.5$. The curves labeled C correspond to path C where the simulations took 50 ps with $\mu = 0.25$.

TABLE III. Free energy associated with the mutation of molecule X to Y (forward) and molecule Y to X (reverse) via path B in Fig. 2. Stages I, II, and III correspond to the three stages shown in Fig. 2.

	Time (ps)	Forward (kJ mol^{-1})	Reverse (kJ mol^{-1})	Average[a] (kJ mol^{-1})
Stage I	10	7.57	7.13	7.35 ± 0.22
	20	7.39	7.23	7.31 ± 0.08
	50	7.40	7.26	7.33 ± 0.07
Stage II	10	1.59	−0.41	0.59 ± 1.0
	20	0.84	−0.62	0.11 ± 0.73
	50	0.64	0.04	0.34 ± 0.30
	100	0.39	0.27	0.33 ± 0.06
Stage III	10	−5.97	−6.14	−6.06 ± 0.08
	20	−6.05	−6.14	−6.09 ± 0.04
	50	−6.08	−6.04	−6.06 ± 0.02
Total	10 + 10 + 10			1.88 ± 1.3
	20 + 20 + 20			1.33 ± 0.8
	50 + 50 + 50			1.61 ± 0.39
	50 + 100 + 50			1.60 ± 0.15

[a] Error is half the hysteresis between the forward and reverse processes.

Y the expected *trans:gauche* ratio when $\mu = 1.0$ is approximately 3:61. This ratio changes to 8:56 when $\mu = 0.5$. Scaling the potential by half results in a smaller disruption of the system for molecule Y than for molecule X which reflects itself in the smaller hysteresis in stage III as compared to stage I.

D. Comparison of pathways

Summing the most accurate contributions from each of the three stages yields a value for the overall free energy for the mutation of X to Y of 1.60 ± 0.15 kJ/mol where the error is again given as half the hysteresis between the forward and reverse processes. Including the 20 ps equilibration time between stages I and II, and between stages II and III the total simulation time was under 250 ps. This is to be compared to a value of 1.72 ± 1.2 kJ/mol calculated via pathway A over 500 ps. Thus by choosing a more appropriate path we have been able to simultaneously reduce the simulation time by half and the observed hysteresis by almost 1 order of magnitude. The overall improvement in the reversibility of the pathway can be seen dramatically in Fig. 7 which again shows curves corresponding to the mutation via pathway A over 500 ps with the solid line representing the forward process and the broken line the reverse process. Above these lie the corresponding curves for pathways B and C plotted as a composite of stages I, II, and III. As can be seen, there is very little divergence between the forward and reverse processes at any stage in pathways B and C indicating that the process is in fact carried out reversibly. It can also be seen that the bulk of the observed hysteresis is generated during stage I, the initial downscaling of the potential. As each stage is fully independent it would be possible by repeating stage I with a longer simulation time to reduce the hysteresis still further.

The overall efficiency of the path depends upon the sum

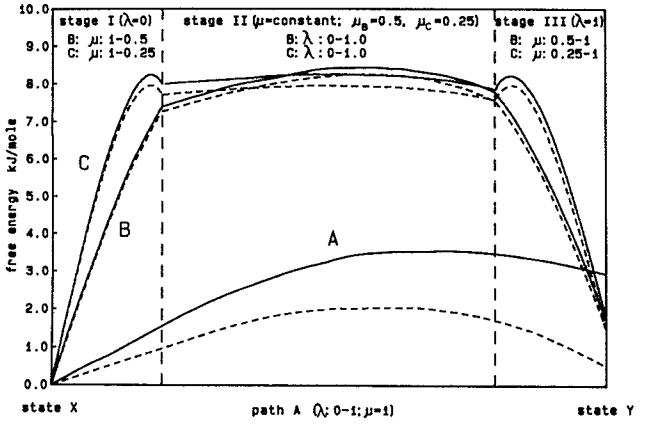


FIG. 7. A comparison of the calculated relative free energy for the mutation of molecule X to Y (solid lines) and for the reverse mutation Y to X (broken lines) for each of the three pathways shown in Fig. 2. The relevant pathway is indicated adjacent to each curve and all curves are referenced to $\Delta A = 0$ when $V_{\text{eff}} = V_x$. For path A the relative free energy has been plotted as a function of the coupling parameter λ (lower scale). Pathways B and C are presented as composites of stages I, II and III. They are presented as a function of the scaling factor μ and the coupling parameter λ as indicated by the appropriate upper scale.

TABLE IV. Free energy associated with the mutation of molecule X to Y (forward) and molecule Y to X (reverse) via path C in Fig. 2.

	Time (ps)	Forward (kJ mol^{-1})	Reverse (kJ mol^{-1})	Average[a] (kJ mol^{-1})
Stage I	10	8.32	7.08	7.70 ± 0.62
	20	8.07	7.45	7.76 ± 0.31
	50	7.98	7.70	7.84 ± 0.14
Stage II	10	0.28	−0.06	$+0.11 \pm 0.17$
	20	−0.17	−0.13	-0.15 ± 0.02
	50	−0.14	−0.10	-0.12 ± 0.02
Stage III	10	−7.03	−6.53	-6.78 ± 0.25
	20	−6.09	−6.47	-6.28 ± 0.19
	50	−6.05	−6.14	-6.10 ± 0.05
Total	10 + 10 + 10			1.03 ± 1.0
	20 + 20 + 20			1.33 ± 0.52
	50 + 50 + 50			1.62 ± 0.21

[a] Error is half the hysteresis between forward and reverse processes.

of each of the three stages. Decreasing μ from $\mu = 0.5$ (path B) to $\mu = 0.25$ (path C) further reduces the time required to obtain low hysteresis for stage II ($\lambda = 0$ to $\lambda = 1.0$) as expected. However, this gain is offset by the increase in time required to scale the potential reversibly. This can be seen by comparing the hysteresis from stages I and III over 10, 20, and 50 ps for path C shown in Table IV to that for path B given in Table III. The hysteresis for comparable times and stages I and III for path C is over twice that for path B. Lowering the interaction potential increases the rate at which configurational space is sampled. It also increases the total configurational space accessible to the system. Thus scaling the potential does not lead to a monotonic change in free energy as a function of μ: in Fig. 7 the free energy change in stages I and III along path C is not monotonic. As the accessible configurational space increases so does the time required for effective sampling. Depending on the system this can, as we have seen, be compensated by an increase in sampling efficiency as μ is decreased. As the interaction potential approaches zero the problem will be dominated by the size of the accessible configurational space. It would not, therefore, be efficient in a normal system to scale the potential such that $\mu = 0$. This effect can be seen in part in regard to path C. From Fig. 7 it is obvious that the major component of the hysteresis in path C occurs during the scaling of the potential in stages I and III as $\mu \rightarrow 0.25$.

Although path C may not be optimal, it is nevertheless, significantly more efficient than path A. Most importantly both path B and C yield essentially the same final result of $\Delta A = 1.6 \pm 0.2$ kJ mol^{-1} for the mutation of molecule X into Y.

The optimal value of the scaling factor μ will vary from system to system. It will depend on the primary relaxation of the system with respect to the mutation of interest. As scaling the potential introduces extra steps with associated errors the minimum deviation of μ from the value 1 required to allow the mutation of interest to be reversibly simulated in the available computer time should normally be chosen.

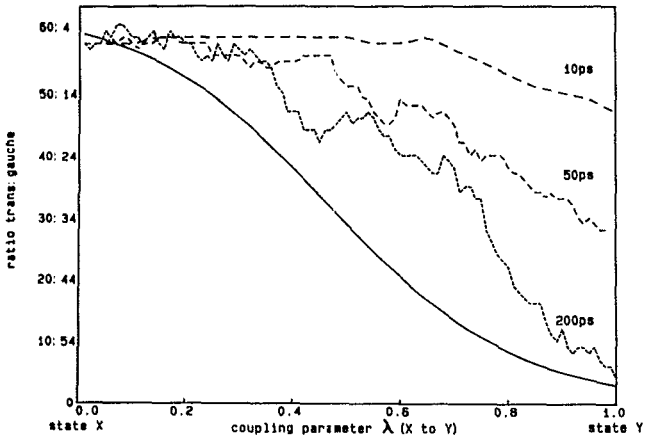


FIG. 8. Ratio of *trans* to *gauche* conformations as a function of λ and time during the simulations along pathway A in which the molecules were changed from state X to state Y. The solid line is an analytical approximation based on Eqs. (9) and (16).

V. DISCUSSION

A prerequisite of application of the coupling parameter technique to determine the relative free energy difference between two states of a system is that the system remains continuously at or close to equilibrium for each value of the coupling parameters λ and μ. In our model system the equilibrium distribution in terms of the ration of *trans* to *gauche* conformations is approximately known from Eqs. (9) and (16) for each value of λ. This ratio is shown in Fig. 8 (solid curve). The actual development of the *trans:gauche* ratio during the simulations along pathway A in which λ is changed from 0 to 1 is also displayed in Fig. 8. When the potential energy function is rapidly (10 ps) changed from state X to state Y, the *trans:gauche* ratio cannot nearly follow fast enough, so the system is not in equilibrium during the change. The slower the change is carried out the closer the system remains near the ideal *trans:gauche* ratio, but even for a 200 ps period of change, the actual ratio is still lagging the equilibrium ratio.

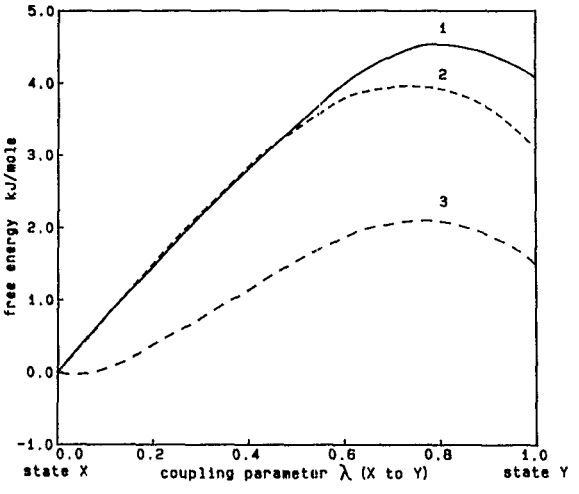


FIG. 9. Relative free energy as a function of the coupling parameter λ for the mutation of molecule X to Y via path A over 100 ps showing the effect of different starting configurations of the system. The ratio of *trans:gauche* conformers in the starting configuration is curve 1 60:4; curve 2 58:6; and curve 3 5:59.

If the system is required to be in equilibrium at each point of the trajectory, this condition is also critically important to the starting configuration. The effect that different starting configurations can have on the free energy change is illustrated in Fig. 9. It shows a plot of the relative free energy vs the coupling parameter λ for the direct mutation (path A) over 100 ps of molecule X to molecule Y for three simulations (labeled 1, 2, and 3) starting from three different initial configurations of the system. Curve 1 in Fig. 9 is identical to the uppermost curve in Fig. 4, its starting configuration 60 *trans* and 4 *gauche* molecules. For curve 2 the ratio was 58 *trans* to 6 *gauche*. The starting configurations for curves 1 and 2 are instantaneous configurations of the system selected at random from an equilibrium simulation of molecule X. They both represent possible configurations of the system in equilibrium. The difference in the final results for the free energy is, however, almost 1 kJ/mol (see also Table II). This is of the same order of magnitude as the total free energy change between molecule X and Y.

Curve 3 in Fig. 9 illustrates an extreme case where the starting configuration contained 5 *trans* and 59 *gauche* molecules. In this case $\Delta A_{X\to Y} = 1.48$ kJ mol^{-1}, a small value due to the fact that relatively little work is to be done when changing from X to Y in this case as the initial configuration of the system is taken from an equilibrium ensemble belonging to Y. If we compare this result to the values given in Table II for the reverse process from molecule Y to X it is obvious that the final value for a free energy change and the observed hysteresis can be highly dependent on the starting configuration. As the length of the simulation is increased the final result is progressively less dependent on the starting configuration. This can be seen by comparing the two entries in Table II for the mutation from molecule X to Y over 200 ps. The two entries correspond to the same starting configurations as used in curves 1 and 2 in Fig. 9. The discrepancy in the forward integration results over 200 ps is only 0.1 kJ mol^{-1} as compared to 1 kJ mol^{-1} over 100 ps of simulation. In 200 ps configurational space is sampled better. Table II also highlights the danger of the assumption that a correct value for the free energy can be simply obtained by taking the average of the free energy change for the forward and reverse processes over short simulation times. For the assumption to be true it is necessary that the relaxation mechanisms in the forward and reverse processes are identical. This is only necessarily true as the system approaches equilibrium. When the system is perturbed at a rate greater than τ_{system} it will no longer remain in equilibrium and an equilibrium distribution of configurations will not be sampled. It is obvious from Figs. 8 and 9 that in this case the calculated free energy and hence the hysteresis between the forward and reverse processes will be highly dependent on the exact configuration of the system. Taking one or the other of the two values for the free energy change in the forward direction given in column two of Table II would yield substantially different values for

the total free energy change at short simulation times. In Table IV it can be seen that the average of the forward and reverse mutations over a total of 30 ps is significantly different from the average of the same mutation over 150 ps.

It should also be remembered that the model system chosen for this study consists of 64 identical molecules. If we considered a single molecule of X in isolation averaged over time there would be a predominance of the *trans* conformation over the *gauche* in a ratio of approximately 15:1. At any given instant, however, the molecule can be either trans or gauche. Therefore, no matter which single conformation is chosen as a starting structure an equilibrium distribution will not be sampled. This is unless the length of the simulation is sufficient to allow a representative proportion of possible conformations to be sampled at every value of λ. Thus it is not normally possible to draw any conclusions as to the efficacy of a result from a free energy calculation on the basis of a single simulation.

VI. CONCLUSIONS

In this paper we have demonstrated that it is possible to significantly reduce the time required to determine the relative free energy between two states of a system by choosing a pathway that minimizes the relaxation time of the system τ_{system}, in response to the change from one state to the other. A general method to reduce τ_{system} has been presented. It involves reduction of parts of the interaction potential of the system, before the system is changed from one state to the other. Scaling down the potential increases the rate at which configuration space is sampled and by reducing potential energy barriers reduces τ_{system}. The system can thus be changed more rapidly while still maintaining equilibrium. The process is completed by rescaling the potential to the original level after the change has been made. Although such a path is indirect and the absolute change in free energy may be large we have shown that the observed hysteresis in the free energy calculated for the forward and reverse processes can be greatly reduced.

The optimum value of the scaling factor μ will depend on the type of system. This is because the increase in the rate at which the system can be changed as μ is reduced, must be weighted against the time required to scale the potential energy function reversibly. This time may be reduced in part by only scaling those regions of the potential energy function which directly influence τ_{system}. When considering a mutation of a protein one may think of a reduction of the interaction between atoms within a given distance from the mutated sidechain.

This work has also demonstrated to what extent the calculated value for the free energy change may depend on the initial configuration of the system. This has highlighted the requirement that the simulation starts from an equilibrium distribution and the danger of basing conclusions on the endpoint free energy of a single simulation. We have also shown that a simple average of the free energy change for the forward and reverse processes calculated over a same time period does not reliably yield the correct free energy difference between two states nor can the final state of the system be used to assess whether the system has remained in equilibrium throughout the simulation.

ACKNOWLEDGMENTS

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